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Full Length Research Paper

The relation between *IL-6*- 174 *G>C promoter* polymorphism and risk of prostate cancer

Nega Berhane

Department of Biotechnology, University of Gondar, POB 196 Gondar, Ethiopia
Email: tesnega@yahoo.com

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Prostate Cancer (PCa) is progressively more common diagnosis in Western societies and in those following Western lifestyles and diets. Despite progresses in prevention and early detection, refinements in surgical technique and improvements in adjuvant radio-therapy and chemotherapy, the aptitude to cure many patients with prostate cancer remains elusive. Various efforts have been made to assess the effects of cytokine gene polymorphisms on the risk of prostate cancer among different population. Interleukin-6 (*IL-6*) has an important role during prostate cancer progression and there has been reports that the IL-6 levels in the serum of patients with hormone refractory and metastatic prostate cancer are significantly increased compared with those in patients with hormone sensitive and localized prostate cancer. However, the result regarding its risk is yet to be comprehensive. The aim of the present study was to evaluate *IL-6* – 174 G>C polymorphism and risk of PCa among 150 prostate cancer patients and equal number of age matched control groups. For this case control study 150 prostate cancer patients, 150 BPH (benign hyperplasia) and 150 health controls were recruited. DNA was isolated by Phenol, chloroform method and the PCR-RFLP (Polymerase chain reaction- Restriction Fragment length Polymorphism) was employed to amplify and create a restriction site on the gene of interest. There was statistically increased risk of PCa among cases (OR= 1.77, 95%CI, 0.65-4.91, p<0.05) who contained the *CC* genotype when compared with health controls. Moreover, the same genotype has elevated the relative risk (OR, 1.63, 95% CI, 1.01-2.62, *p*<0.05) when smoking has been take into account.

Keywords: BPH; IL-6; polymorphism; prostate cancer;

INTERODUCTION

Among men, prostate cancer has a high prevalence, with relatively lower cancer-specific mortality risk compared to lung and colon cancer. Prostate-specific antigen (PSA) screening has increased prostate cancer awareness since its implementation as a screening tool almost 25 years ago, but, due to the largely indolent course of this disease and the unspecific nature of the PSA test, increased incidence has largely been observed (Barqawi *et al.,* 2012). Accounting for 29% of all cancers in men, prostate cancer is the most common cancer among men behind nonmelanoma skin cancer and is the second highest cause of cancer death among men of all races (Jemal *et al.,* 2010, Siegel *et al.,* 2011). Interleukin-6 (*IL-6*) is an immunoregulatory pleiotropic cytokine that activates a cell-surface signaling assembly composed of IL6, IL6RA, and the shared signaling receptor gp130 (Boulanger *et al.,* 2003). The cytokine is involved in different physiological and pathophysiological processes,

such as inflammation, bone metabolism, synthesis of CRP (C-reactive protein), and carcinogenesis (Diehl and Rincón, 2002). The gene encoding *IL-6* is localized at chromosome 7p21–14.1 (Akira, 1993). It is a glycoprotein consisting of 212 amino acids. The expression of IL-6 and its receptor has been consistently demonstrated, not only in human prostate cancer cell lines, but more importantly in human prostate carcinoma and benign prostate hyperplasia, while tissue specimens from patients with organ confined prostate cancer have higher IL-6 and IL-6 receptor levels (Hobisch *et al.,* 2000; Giri *et al.,* 2001) Multiple studies have demonstrated that IL-6 is increased in the serum of patients with metastatic prostate cancer and IL-6 levels correlate with tumor burden as well as with serum prostate specific antigen (PSA) or clinical evident metastases (Alder *et al.,* 1999; Drachenberg *et al.,*1999). A common *G>C* polymorphism at position -174 of the interleukin-6 (*IL-6*) gene promoter is thought to affect the expression level of this gene, thus predisposing to a variety of diseases, including tumorigenesis (Litovkin *et al.,* 20007*).* Interleukin-6 (IL-6) is a proinflammatory cytokine produced by a number of cell types including cancer cells (Conze *et al.,* 2001). Elevated levels of circulating interleukin-6 have been seen in many clinical situations characterized by tissue injury, such as trauma (including major surgery), burns, malignant conditions, exposure to toxins and aseptic irritants, immune hypersensitivity reactions, inflammatory, infectious and autoimmune diseases (Papanicolaou *et al.,* 1998). As a growth factor IL-6 plays a significant role in cell differentiation and is believed to be involved in tumor progression (Hirano*,* 1998). High IL-6 levels have been shown to correlate with poor prognosis and high mortality in prostate and colorectal cancer patients (Nakashima *et al.,* 2000; Chung *et al*., 2003). Cytokine polymorphisms, possibly associated with differential cytokine production, are risk factors for recurrent prostate cancer. The 174 *IL-6* G>C genotype appears to be biologically and clinically important. There is increasing evidence that *IL-6* gene polymorphism is associated with altered levels of IL-6 expression (Tan *et al.,* 2005). Therefore, the present study aimed to assess the effect of this gene polymorphism towards risk of prostate cancer in north Indian population.

MATERIALS AND METHODS

Blood samples from 150 north Indian males with prostate cancer were collected in sterile EDTA-K2 coated non-vacationous tubes (Becton–Dickinson, San Jose, CA, USA). The samples were collected at the Departments of Urology of the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, the All India Institute of Medical Sciences (AIIMS), New Delhi and Government Medical College and Hospital, Patiala, Punjab in north India. All patients were histologicaly diagnosed carcinoma of the prostate confirmed with biopsy studies as well as prostate specific antigens (PSA) test. There were 300 age matched control samples, of which 150 were free from any symptom of disease, whereas the rest 150 were diagnosed with benign prostate hyperplasia (BPH). All the samples were not originated from the same hospital; however, all of the individuals recruited for this study were in the same geographical area. None of the patients were given either chemo or radiotherapy before providing the sample. The samples were collected by the clinical staff of the hospitals concerned. A detailed questionnaire designed by the Indian Council of Medical Research (ICMR) encompassing details of the disease diagnosis, family history, age, smoking and drinking status was completed at the time of collection of the samples. Besides, the pathological grading and staging of the cancer was confirmed from the hospital record. Blood samples were stored at -80^{0}C till extraction of DNA. Written informed consent was obtained from all cases and controls. The study was carried out after obtaining approval from the Ethics Committee of the PGIMER, Chandigarh, India.

Genotyping of *IL-6- 174 G>C*

Genomic DNA was extracted from peripheral blood lymphocytes by standardized protienase K digestion and phenol– chloroform extraction. A PCR- RFLP technique was utilized to amplify *IL- 6* - 174 *G>C* polymorphism according to the protocol given by Sery *et al.* (2003). The primer sequences used to amplify 204 bp PCR products were, forward 5'-ACTTTTCCCCCTAGTTGTTTCTTTC-3' and reverse 5'-AGACATGAGCCTCAGACATCTCAGT-3'. The PCR reaction was performed in a total volume of 50µl containing 200ng of genomic DNA, 200µM of each dNTP, 50mM KCl, 1mM each of the primers and 1.5 U of Taq polymerase. (Sigma Alderich, USA). The PCR conditions utilized to amplify the gene were an initial denaturation step of 5min at 95°C coupled to 35cycles of 45s at 95°C, 30s at 55°C and 45s at 74 °C, followed by 74 °C for 6 min that amplified 204 bp PCR products (Fig. 1). RFLP of *IL 6* was performed in a total reaction volume of 10µl, where 2µl of 204 bp PCR product, 1.8µl of buffer, 8 U of TaqI restriction enzyme and 5.2µl of distilled water were mixed together and incubated for 5 hours at 65^{o}C. The digested product was analyzed by 2.5% agarose gel electrophoresis containing ethidium bromide. The 204 bp product was cleaved in two fragments of 180 and 24 bp by Taq I enzyme. The uncut product of 204 bp identified *CC* and the cut fragment identified *GG* genotype. The heterozygous *GC* genotype was identified by the presence of both 204 and 180 bp products (Fig. 2).

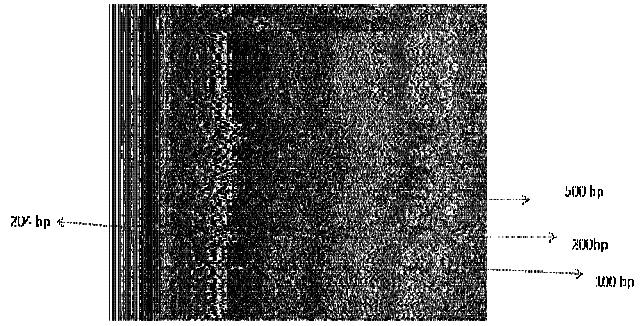

Fig 1 204 bp PCR amplification product of IL-*6*-174 *G>C* gene
Lanes 1-7, 204 bp PCR product
Lane 8 100 bp DNA marker

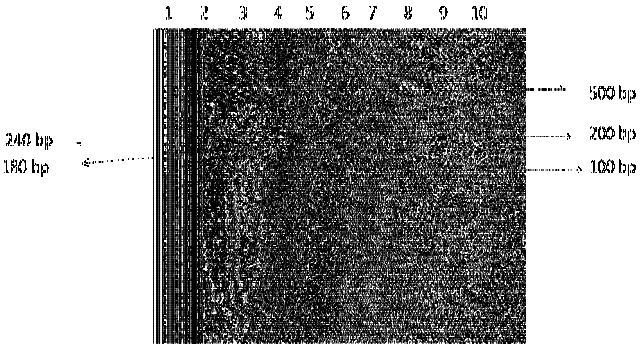

Fig 2 representative agarose gel electrophoresis of IL-*6* gene after digestion with Taq I enzyme
Lane 10 100 bp DNA ladder
Lanes 1,3,5,6, and 9 heterozygous *GC* 204,180 bp
Lanes 2 and 8 homozygous *CC* 204 bp
Lanes 4 and 7 Homozygous *GG* 180 bp

Table 1 *IL-6* 174 *G-C* genotype frequency, OR and 95% CI for prostate cancer among cases and controls.

Genotype	Cases	Controls	OR (95% CI)
GG	82 (54.7%)	84 (56.0%)	1.0 Ref.
GC	54 (36%)	58 (38.7%)	0.94 (0.57-1.57)
CC	14 (9.3%)	8 (5.3%)	1.77 (0.65-4.91)
Allelic frequency			
G	218	226	1.0 Ref.
C	82	74	1.14 (0.78-1.67)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (centre for disease control and prevention).

Statistical Analysis

Relevant data of cases, BPH and health controls such as age, inhabitancy, occupation, smoking, stage of the disease and drinking habits were tabulated. The effect and association of the I polymorphism on risk of PCa was analyzed by computing odds ratio (OR) and 95 % confidence interval (CI). The statistical analysis was performed using Epi-Info software (Epi-Info, version 3.5.1. Center for Disease Control and Prevention, Atlanta, GA, USA, August 13, 2008) and software SPSS version 11.5 (SPSS, Chicago, IL). Chi square test was utilized to check for the Hardy–Weinberg's equilibrium. Significance was set at $p<0.05$.

RESULTS

Study Population

The Demographic characteristics of study subjects were reported elsewhere. There were no significance difference in the age of cases, BPH and health controls.

Table 2 *IL-6* -174 *G-C* genotype frequencies and OR and 95% CI for prostate cancer among cases and BPH.

Genotype	Cases	BPH	OR (95% CI)
GG	82 (54.7%)	80 (53.3%)	1.0 Ref.
GC	54 (36%)	60 (40%)	0.80 (0.53-1.46)
CC	14 (9.3%)	10 (6.7%)	1.37 (0.80-1.67)
Allelic frequency			
G	218	220	1.0 Ref.
C	82	80	1.03 (0.71-1.51)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (center for disease control and prevention).

Table 3 Risk of prostate cancer in smokers due to *IL-6*-174 *G-C* polymorphisms among cases and controls.

Genotype	Smokers		OR (95% CI)	Non smokers		OR (95% CI)
	cases	Controls		cases	Controls	
GG	26 (17.3%)	24 (16%)	1.16 (0.57-2.38)	56 (ref.) (37.3%)	60 (ref.) (40%)	1.0
GC	18 (12%)	15 (10%)	1.29 (0.55-3.00)	36 (24%)	43 (28.7%)	0.97(0.44-2.10)
CC	4 (2.7%)	1 (0.7%)	1.66 (1.03-2.67)	10 (6.7%)	7 (4.7%)	1.20 (0.52-2.80)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (center for disease control and prevention).

Table 4 Risk of prostate cancer in smokers due to *IL-6*-174 *G-C* polymorphisms among cases and BPH.

Genotype	Smokers		OR (95% CI)	Non smokers		OR (95% CI)
	cases	BPH		cases	BPH	
GG	26 (17.3%)	22 (14.7%)	1.22 (0.59-2.54)	56 (ref.) (37.3%)	58 (ref.) (38.7%)	1.0
GC	18 (12%)	13 (8.7%)	1.43 (0.60-3.45)	36 (24%)	47 (31.3%)	0.58 (0.39-1.85)
CC	4 (2.7%)	1 (0.7%)	1.63 (1.01-2.62)	10 (6.7%)	9 (6.0%)	0.97 (0.40-2.33)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (center for disease control and prevention).

The study comprised both rural and urban as described previously (Berhane *et al.,* 2012). In this case control study sedentary, manual and industrial workers were involved. The genotype frequency of *IL-6* G>C polymorphism in cases and controls is describe in Table 1. The percentage of *GG* genotype in cases was 54.7 unlike 56 percent in controls. The percentage of the heterozygous *GC* genotype in cases was 36 and it was 38 in controls. The percentage of the homozygous variant *CC* genotype in cases was 9.3% unlike 5.3 % in controls. The *G* allele frequency was 0.73 in cases and it was 0.75 in controls. On the other hand the *C* allele frequency was 0.27 in cases and it was 0.25 in controls. Statistically significant 1.77 folds of increased risk of prostate cancer was observed in cases due to the *CC* genotype of *IL- 6* (OR=1.77, 95% CI=0.65-4.91, $p<0.05$).

The frequency of the three genotypes of *IL-6* among cases and BPH study subjects is given in Table 2. The percentages of *GG*, *GC* and *CC* genotypes in BPH study subjects were 53.3, 40 and 6.7, respectively. There was no statistically significance association towards risk of prostate cancer among cases when it is computed with BPH study subjects.

The relative risk of prostate cancer due to smoking and *IL-6* gene polymorphism among cases and controls is given in Table 3. The percentages of the three genotypes among smoker cases were 17.3, 12 and 2.7 for *GG*, GC and *CC,* respectively. OR and 95% CI of smoker cases was computed with healthy non smoker controls. 1.66 folds of statistically significant increased risk of prostate cancer was association due to smoking for *CC* genotype carriers (OR=1.66, 95% CI=1.03-2.67, $p<0.05$).

The relative risk of prostate cancer due to smoking and *IL-6* 174 *G-C* gene polymorphism among cases and BPH study subjects is given in Table 4. The genotype frequency of smokers in BPH subjects was 14.7 *GG*, 8.7 *GC* and 0.7 percent *CC*. There was statistically no significant association with smoking and any of *IL-6* gene polymorphisms when smoking as a risk was compared against BPH non smoking study subjects.

The effect of alcohol and *IL-6* polymorphism towards risk of prostate cancer among cases and controls is given in Table 5. The percentages of the three genotypes of *IL-6* among cases were 24.7 *GG*, 14 *GC* and 2.7 *CC*. The relative risk of alcohol and *IL-6* 174 *G>C* polymorphism of cases was computed against non drunker healthy

Table 5 Risk of prostate cancer in drinkers due to *IL-6*-174 *G-C* polymorphisms among cases and controls.

Genotype	Alcoholic		OR (95% CI)	Non drinkers		OR (95% CI)
	cases	Controls		cases	Controls	
GG	37 (24.7%)	30 (20%)	1.21 (0.90-1.65)	45(ref.) (30%)	54(ref.) (36%)	1.0 Ref.
GC	21 (14%)	27 (18%)	0.931.98) (0.44-	33 (22%)	31 (20.7%)	0.99 (0.47-2.10)
CC	4 (2.7%)	4 (2.7%)	1.10 (0.53-2.27)	10 (6.7%)	4 (2.7%)	1.23 (0.59-2.57)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (Centre for disease control and prevention).

Table 6 Risk of prostate cancer in drinkers due to *IL-6*-174 *G-C* polymorphisms among cases and BPH.

Genotype	Alcoholic		OR (95% CI)	Non drinkers		OR (95% CI)
	cases	BPH		cases	BPH	
GG	37 (24.7%)	31 (20.7%)	1.14 (0.84-1.54)	45 ((ref.) 30%)	49 (ref.) (32.7%)	1.0 Ref.
GC	21 (14%)	27 (18.0%)	0.85 (0.40-1.81)	33 (22%)	33 (22%)	0.84 (0.40-1.79)
CC	4 (2.7%)	4 (2.7%)	1.04 (0.51-2.16)	10 (6.7%)	6 (4%)	0.93 (0.42-2.06)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (Centre for disease control and prevention).

controls. There was statistically no significant association to any of the genotypes of *IL-6* alcoholism and risk of prostate cancer.

The OR and 95 % CI of *IL-6* 174 *G-C* polymorphism and effect of alcohol among cases and BPH subjects were computed in Table 6. There were 20.7% *GG*, 18% *GC* and 2.7% *CC* genotype carriers among alcoholic BPH study subjects. The effect of alcohol in risk of prostate cancer among cases was computed against non alcoholic BPH subjects. There was statistically no significant association between alcohol and polymorphism of *IL-6* genotypes when compared to BPH non alcoholic study subjects.

DISCUSSION

Interleukin-6 (IL-6) is a pleiotropic growth factor involved in many physiological and pathological processes including carcinogenesis (Ishihara and Hirano, 2002). Chronic inflammation plays a role in transformation from normal cell to malignant state. Interleukin-6 (*IL-6*) regulates inflammation and various physiological processes. *IL-6* promoter polymorphism (–174G>C) is associated with transcription differences *in vitro* and *in vivo*. High expression of IL-6 may result in oxidative DNA damage and enhance risk of carcinogenesis (Upadhyay *et al*., 2008). High serum levels of IL-6 have been associated with advanced stage disease and worse prognosis for several cancer types including ovarian, breast and colorectal (Berek *et al*., 1991; Zhang and Adachi, 1999; Belluco *et al.,* 2003). In contrast, however, high levels of IL-6 protein and mRNA expression within the breast carcinoma tissue have been linked to better prognosis and to a less malignant phenotype (Basolo *et al*., 1996). A common *G>C* polymorphism located within the *IL-6* promoter at position -174 has been reported to influence IL-6 expression, with the G allele being associated with higher expression levels (Fishman *et al*., 1998; Terry *et al.,* 2000; Vickers *et al.,* 2002). This polymorphism has been implicated in a number of chronic disease conditions including arthritis, coronary heart disease and diabetes (Fishman *et al*., 1998; Fernandez-Real *et al.,* 2000). In human cancer, the _174 G/C IL-6 polymorphism does not appear to be a risk factor for the development of multiple myeloma or melanoma (Zheng *et al.,* 2000). Data, however, suggest that the *C* allele is associated with an increased risk of colorectal cancer (Landi *et al*., 2003). Furthermore, in ovarian cancer, the *C* allele is associated with an earlier stage of disease and with significantly better survival (Hefler *et al*., 2003).

In the present study this important polymorphism was evaluated for risk of prostate cancer in 150 prostate patients from north Indian population. There was statistically significance association between this polymorphism and risk of prostate cancer. Similar results has been reported by Dominique *et al*. (2006) and Xu *et al*. (2005) who found a direct relationship between polymorphisms of IL-6–174*G>C* and risk of prostate cancer in European and American people. However, Bao *et al*. (2008) found null results in Han people living in Hubei regions, China. We believe that the discrepancy might be ascribed, to the fact that the polymorphism of –174*G/C* has ethnic difference. The results of this study are not consistent with the findings of other related studies in China (Zhai et *al*., 2001 and Liu *et al*., 2005), which further suggests that the polymorphism of –

174*G>C* in Chinese is different from that of European and American people but is close to that of oriental people such as Japanese and Korean (Lim *et al.,* 2002).

Despite prostate cancer the association of *IL-6* –174 *G>C* polymorphism with risk of several malignancies and inflammatory diseases such as juvenile chronic arthritis (Fish man *et al*., 1998), Kaposis sarcomas, (Foster *et al*., 2000), colorectal cancer (Bullcuo *et al*., 2003), ovarian cancer (Hefler et al., 2003), Hodgkin lymphoma (Cozen *et al.,* 2004), non small cell lung cancer (Campa *et al*., 2004), prostate cancer (Tan *et al.,* 2005), and peripheral arterial disease has been reported for different ethnic background.

Elevated levels of IL-6 in serum and malignant tissues have also been shown to be associated with poor prognosis of esophageal cancer (OKa *et al.,* 1996; Wang *et al*., 1999). The reason behind the association of IL-6 genotype with susceptibility of esophageal cancer may be due to blocked cytotoxic function of tumor-infiltrating lymphocytes by high local IL-6 concentrations at tumor sites (Tanner *et al.,* 1999). Thus, it is possible that increased IL-6 production in –174C non-carriers may lead to escape of esophageal tumor cells from immune surveillance and promote oncogenesis (Oka *et al*., 1996). Earlier studies also showed association of elevated serum IL-6 level with esophageal tumor stage, lymph node metastasis and with poor prognosis (Wang *et al*., 1999). The persistence of the pro-inflammatory cytokine IL-6 response maintains chronic recruitment of lymphocytes and mast cells which stimulates schemas cell carcinoma invasion and metastasis. Once carcinoma *in situ* penetrates the basement membrane to involve the lamina propria, it is invasive carcinoma and capable of widespread dissemination. Therefore, understanding the interplay of genes and the pathways they utilize can lead to the detection of novel molecular targets in the diagnosis, prognosis, and treatment of cancers. Environmental factors like smoking and alcohol are well established mediators of high risk in cancer such as esophageal (van Leeuwen *et al.,* 2007). Accordingly, in this study statistically significant additional risk of prostate cancer was observed in cases due to smoking. However, different results for oesophageal cancer was described by Upadhyay *et al.* (2008) who reported in their study that interaction of *IL-6* –174G>C polymorphism with environmental factors (like tobacco usage, alcohol or household combustible fuels) did not modulate the risk of esophageal cancer. This discrepancy might be explained by difference in study design, difference in ethical background and small statistical power.

Similar studies with large sample size are warranted to determine the role of environmental factors like smoking and drinking alcohol and association of IL-6-174 G>C polymorphism and risk of prostate cancer both in the same and different ethnic backgrounds since the role of alcohol and smoking in prostate cancer risk in the literature is not consistence.

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